WHAT WE CLAIM IS:

1. An isolated polypeptide comprising:

   a) an amino acid sequence as set forth in any one of SEQ ID NOs. 1, 3, 5 or 7; or

   b) a functional fragment or variant of the polypeptides in a) above, wherein the fragment or variant provokes a humoral and/or cellular immunological response in an animal with similar characteristics to that produced by a polypeptide as outlined above.

2. An isolated polypeptide as claimed in claims 1 wherein the functional fragment or variant incorporates a B cell or T cell epitope of the polypeptide.

3. An isolated nucleic acid molecule wherein the molecule:

   a) comprises a nucleotide sequence as set forth in any one of SEQ ID NOs. 2, 4, 6 or 8;

   b) is a functional fragment or variant of the molecule(s) in a); or

   c) is able to hybridise under stringent conditions to the molecule(s) in a) or b); or

   d) is a complement of the molecule(s) defined in a), b) or c); or

   e) is an anti-sense sequence corresponding to any of the sequences in a) – d).

4. An isolated nucleic acid molecule encoding a polypeptide as claimed in either claim 1 or 2.

5. A vector or construct comprising the nucleic acid molecule as claimed in claim 4.

6. A host cell which has been transformed with a vector or construct as claimed in claim 5.

7. An isolated ligand which binds to a polypeptide as claimed in either claim 1 or 2.

8. A probe capable of hybridizing under stringent conditions to a nucleic acid molecule as claimed in either claim 3 or4.

9. A probe for a polypeptide as claimed in either claim 1 or 2.

10. A probe for the ligand of claim 7 when the ligand is bound to the polypeptide.

11. A method for determining the immune status of an animal to a nematode infection characterized by steps of:

a) obtaining a blood or serum sample from the animal;

b) preparing an IgE enriched or IgG depleted preparation of the sample in a);

c) contacting the sample at a) with a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs. 1, 3, 5 or 7 or a functional fragment or variant thereof;

d) contacting the preparation from c) with a probe for the immuno-complex formed by IgE and the polypeptide;

e) detecting the probe to identify the immune status of the animal by the presence or absence of the probe.

12. A method for determining the immune status of an animal to a nematode infection characterized by steps of:

a) obtaining a blood or serum sample from the animal

b) preparing an IgE enriched or IgG depleted preparation of the sample in a);

c) exposing the preparation from b) with a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs. 1, 3, 5 or 7 or a functional fragment or variant thereof;

d) washing the preparation from c) to remove any unbound IgE (i.e. IgE that is not bound to the polypeptide;

e) detection of immuno-complex formed by the polypeptide and IgE at step c) with monoclonal antibodies to IgE.

f) detection of IgE with appropriately labeled anti-antibodies.

13. A method of determining the immune status of an animal comprising the steps of:

a) exposing a portion of the animal's skin to a polypeptide comprising the amino acid sequence of SEQ ID NOs. 1, 3, 5 or 7 or a functional fragment or variant thereof;

b) determining the immune status by the presence or absence of an immune or allergic reaction.

14. A method for selectively breeding animals resistant to nematode infection characterized by steps of:

a) determining the immune status of male and female animals;

b) selecting males and females disposed to develop immune resistance to nematodes;

c) using selected animals to breed progeny resistant to said infection.

15. An isolated polypeptide as claimed in either claim 1 or 2 wherein the polypeptide is a functional fragment or variant of SEQ ID NO. 5 having at least 90% homology to SEQ ID NO. 5.

16. An isolated nucleic acid molecule as claimed in claim 3 wherein the molecule is a functional fragment or variant of SEQ ID NO. 6 having at least 94% homology to SEQ ID NO. 6.

17. An isolated polypeptide as claimed in either claim 1 or 2 wherein the polypeptide is a functional fragment or variant of SEQ ID NO. 1 having at least substantially 75% homology to SEQ ID NO. 1.

18. An isolated nucleic acid molecule as claimed in claim 3 wherein the molecule is a functional fragment or variant of SEQ ID NO. 2 having at least substantially 70% homology to SEQ ID NO. 2.

19. An isolated polypeptide as claimed in either claim 1 or 2 wherein the polypeptide is a

functional fragment or variant of SEQ ID NO. 3 having at least 80% homology to SEQ ID NO. 3.

20. An isolated nucleic acid molecule as claimed in claim 3 wherein the molecule is a functional fragment or variant of SEQ ID NO. 4 having at least substantially 70% homology to SEQ ID NO. 4.

21. An isolated polypeptide as claimed in either claim 1 or 2 wherein the polypeptide is a functional fragment or variant of SEQ ID NO. 7 having at least 80% homology to SEQ ID NO. 7.

22. An isolated nucleic acid molecule as claimed in claim 3 wherein the molecule is a functional fragment or variant of SEQ ID NO. 8 having at least 75% homology to SEQ ID NO. 8.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 31427/14 AJC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ2003/000205**	International Filing Date *(day/month/year)* 12 September 2003	Priority Date *(day/month/year)* 13 September 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12N 15/15, C07K 14/81, G01N 33/53		
Applicant [AGRESEARCH]LIMITED et al ovita		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [X] Certain observations on the international application

Date of submission of the demand 13 February 2004	Date of completion of the report 7 September 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **JANE MCHENRY** Telephone No. (02) 6283 2091

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000205

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-44**, as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **45-48,** received on **4 August 2004** with the letter of **4 August 2004**
- [X] the drawings, pages **1-8,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the sequence listing part of the description:
  pages **1-6,** as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [X] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be nonobvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos: **7 and 10**

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **7 and 10**

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-6, 8, 9 and 11-22**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-6, 8, 9 and 11-22**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-6, 8, 9 and 11-22**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

The following documents from the ISR are referred to in this report:

D1 = Shaw R J *et al.* (2003) Int. J. Parasitol. 33: 1233-1243.

D2 = De Maere V *et al.* (2002) Parasitology 125: 383-391 & EMBL database accession No. CAD10783.

D3 = WO 1998/012563

D4 = WO 1996/032641

D5 = Frank G R *et al.* (1998) J. Parasitol. 84(6): 1231-1236.

D6 = Duffy M S *et al.* (2002) Clin. Diagn. Lab. Immunol. 9(4): 763-770 & EMBL database accession No. AAG50205.

D7 = EMBL database accession number AAA70419.

The invention appears to reside in the identification of polypeptides and nucleic acid molecules encoding aspartyl protease inhibitors of *Trichostrongylus colubriformis, Ostertagia circumcincta* and *Haemonchus contortus* and uses thereof in identifying animals that are innately more disposed to develop immune resistance to nematodes.

Document D1 was published after the filing date of the present application so does not form part of the prior art. This is a document by the inventors that may be useful in understanding the invention.

Document D2 was published after the priority date but before the filing date of the present application. This document would only be relevant with respect to inventive step if the priority date was found invalid.

NOVELTY

Documents D3-D5 disclose the identification of DiT33 aspartyl protease inhibitor from the nematode *Dirofilaria immitis*. The use of this allergen to immunodiagnose heartworm in animals, particularly cats is discussed. DiT33 shares 38%, 44% and 40% identity and 52%, 58% and 54% similarity with the Aspin polypeptides of SEQ ID NO: 1, 5, 7 of the present invention respectively. Therefore claims 1-6, 8, 9, 11-22 are novel in light of any one of D3-D5.

- Continued on supplementary box below -

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

NOTE: Claims 7 and 10 were not searched regarding novelty and inventiveness. Therefore examination of these claims is not required. Despite this the Examiner has made a few comments on the support of these claims.

Claims 7 and 10 are not fully supported by the description. These claims refer to a ligand which binds to a polypeptide of the invention or a probe for the ligand. The invention resides in the identification of the aspartyl protease inhibitors Tco-Aspin, Oc-Aspin and Hc-Aspin and uses thereof in identifying animals innately more disposed to develop immune resistance to nematodes. The Applicant asserts that it would be mere routine to produce ligands to the identified polypeptides. However, the method of identifying such ligands or probes does not produce ligands it only identifies another characteristic of a known compound. These claims are directed to the ligands *per se*. This encompasses known ligands. These ligands are not limited to being used with the polypeptides of the invention and consequently the claims do not comprise the inventive concept. Therefore the claims are not fully supported by the description.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V

Document D6 discloses the identification of an aspartyl protease inhibitor expressed by the nematode *Parelaphostrogylus tenius*. This document teaches that this protein induced a strong immune response in red deer and may be useful for diagnosis of infection. This protease inhibitor shares 74% identity and 84% similarity with SEQ ID NO: 1 of the present invention. Claims 1-6, 8, 9, 11-22 are novel in light of D6.

Document D7 discloses an aspartyl protease inhibitor from the nematode *Dirofilaria immitis*. This sequence shares 42% identity and 57% similarity with SEQ ID NO: 1. Claims 1-6, 8, 9, 11-22 are novel in light of D7.

INVENTIVE STEP

Documents D3-D7 all disclose aspartyl protease inhibitors that have been cloned from nematodes. This polypeptide is clearly expressed by the nematode during infection and is considered a strong allergen for nematode infection. There is no invention in identifying mere homologues of a known protein, their nucleic acid sequences and cloning the nucleic acid sequences into vectors. These are routine procedures in the art. However, the applicant asserts that the present invention provides the unexpected advantage that the claimed aspartyl protease inhibitors, Tco aspin, Oc-aspin and Hc-aspin, have the ability to illicit an IgE response at a significantly higher level in those animals that have an innate predisposition to immunity. These aspartyl protease inhibitors of the present invention show a direct correlation between high levels of IgE and immunity. The applicants assert this is in contrast to other nematode allergens that do not possess an associated correlation between IgE response and protective immunity. Thus, this unexpected advantage appears to render these claimed aspartyl protease inhibitors inventive. Furthermore, none of the prior art documents teach methods for determining whether an animal is inclined to develop immune resistance to nematode infection. Therefore claims 1-6, 8, 9 and 11-23 are considered to comprise an inventive step.